Translation

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Form PCT/IPEA/416
202dg07.wo		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/001430	16.02.2004	21.03.2003
International Patent Classification (IPC) or national classification and IPC		
Applicant		
DEGUSSA AG		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *(sent to the applicant and to the International Bureau)* a total of _____ sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s))
      _____, containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☒ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
Name and mailing address of the IPEA/EP	Authorized officer
Facsimile No.	Telephone No.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/001430

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on translations from the original language into the following language _____, which is the language of a translation furnished for the purposes of:

☐ international search (Rule 12.3 and 23.1(b))

☐ publication of the international application (Rule 12.4)

☐ international preliminary examination (Rule 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

☐ the international application as originally filed/furnished

☒ the description:

pages 1-22 as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

☒ the claims:

nos. 1-21 as originally filed/furnished

nos.* _____ as amended (together with any statement) under Article 19

nos.* _____ received by this Authority on _____

nos.* _____ received by this Authority on _____

☒ the drawings:

sheets 1/4-4/4 as originally filed/furnished

sheets* _____ received by this Authority on _____

sheets* _____ received by this Authority on _____

☒ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, nos. _____

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to sequence listing *(specify)*: _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages _____

☐ the claims, nos. _____

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

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International application No.
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Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1, 3, 9	YES
	Claims	2, 4-8, 10-21	NO
Inventive step (IS)	Claims		YES
	Claims	1-21	NO
Industrial applicability (IA)	Claims	1-21	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

Reference is made to the following documents:

D1: DATABASE EMBL [Online] EBI; 20 July 2001 (2001-07-20) 'Bacillus stearothermophilus glutamyl-tRNAGln amidotransferase subunit C (gatC), glutamyl-tRNAGln amidotransferase subunit A (gatA), and glutamyl-tRNAGln amidotransferase subunit B (gatB) genes, complete cds.' Database accession no. AY040860 XP002281305

D2: KOBAYASHI M ET AL: 'AMIDASE COUPLED WITH LOW-MOLECULAR-MASS NITRILE HYDRATASE FROM RHODOCOCCUS RHODOCHROUS J1. SEQUENCING AND EXPRESSION OF THE GENE AND PURIFICATION AND CHARACTERIZATION OF THE GENE PRODUCT' EUROPEAN JOURNAL OF BIOCHEMISTRY, BERLIN, DE, Vol. 217, 1993, pages 327-336, XP000652066 ISSN: 0014-2956

D3: D'ABUSCO ANNA SCOTTO ET AL: 'Molecular and biochemical characterization of the recombinant amidase from hyperthermophilic archaeon Sulfolobus solfataricus' EXTREMOPHILES, Vol. 5, No. 3, June 2001 (2001-06), pages 183-192, XP002281301 ISSN: 1431-0651

D4: DATABASE GENBANK PROTEIN [Online] NIH; 6 June 2002

(2002-06-06) PARKHILL, J. ET AL.: 'Putative DNA helicase [Salmonella enterica subsp. Enterica serovar Typhi]' Database accession no. CAD06784 XP002281306 & PARKHILL, J. ET AL.: 'Complete genome sequence of a multiple drug resistant salmonella enterica serovar typhi CT18' NATURE, Vol. 413, 25 October 2001 (2001-10-25), pages 848-852, XP002965014

The present application relates to a gene and protein of an amidase isolated from the thermophile bacterium *Pseudonocardia thermophila*.

D1 describes a glutamyl-tRNA amidotransferase (amidase family) from *Bacillus stearothermophilus*. The subunit gatA has 59% nucleic acid identity with SEQ 4 and 40% amino acid identity with SEQ 3. The amino acids 447-460 from gatA have 86% identity with SEQ 2. Thus, D1 deprives claim 2, and consequently also dependent claims 4-8 and 10, of novelty. With regard to claims 5-7, attention is drawn to the fact that these claims do not characterise the enzyme any further, since the origin could be recombinant in nature, e.g. not limited to that which can actually be isolated from a given wild-type species. D2 describes an amidase of *Rhodococcus rhodochorus* J1 (gene and protein). The latter has 70% nucleic acid identity with SEQ 4 and 67% amino acid identity with SEQ 3. The amino acids 473-485 have 78% identity with SEQ 2. The enzyme enantioselectively converts amides to S-acids, has an optimum temperature of 55 °C and pH stability of 6.7-10. Consequently, D2 deprives claims 2, 4-8 and 10-21 of

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

novelty. D3 discloses an amidase of *Sulfolobus solfataricus* J1 (gene and protein). The latter has 50% amino acid identity with SEQ 3. The amino acids 474-487 have 79% identity with SEQ 2. The enzyme enantioselectively converts amides to S-acids, has an optimum temperature of 95 °C and an optimum pH of 7.5. Various reactions are carried out at 70 °C. Consequently, D3 deprives claims 2, 4-8, 10-12 and 14-21 of novelty.

In conclusion, claims 2, 4-8 and 10-21 are not novel over D1-D3 and therefore do not meet the requirements of PCT Article 33(2). Consequently, they do not satisfy the criterion of inventive step either (PCT Article 33(3)).

Claims 1, 3 and 9 are formally novel over D1-D3 (however, see the comments in Box VIII). However, since they do not sufficiently characterise the enzyme of the present application (see Box VIII), they cannot be deemed inventive (contrary to PCT Article 33(3)).

Box No. VIII **Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claim 1 attempts to characterise the enzyme of the present application by a homology of 50% to a 9-long amino acid sequence from the N terminus of the present amidase. Firstly, the term "N-terminal sequence" used here is unclear since it could relate both to a sequence from the N-terminal half of the protein and also to the amino acid sequence of the N-terminal end. Secondly, it is not clear whether such a definition, which encompasses numerous possibilities, but for which merely one example is given in the description, is sufficient to allow a person skilled in the art to carry out the invention across the entire range claimed, e.g. immediately to provide, on the basis of the description, enzymes with an amidase function which have merely one N-terminal sequence having 50% homology (! not identity) with SEQ 1 but which otherwise are not defined. Moreover, it is apparent from the description that such an amidase also has the properties of the one that was actually isolated, that is, it gives the enzyme these properties. Consequently, claim 1 is not only unclearly worded but also insufficiently supported and disclosed, contrary to PCT Articles 5 and 6. This is particularly clear from D4, which describes a putative DNA helicase from *Salmonella enterica* which has the sequence HMPDPD in amino acids 131-136, which amounts to 6 identical amino acids from 9 of SEQ 1, e.g. 67% identity (! not homology).

Claim 2 is subject to the same defect as claim 1, especially since sequences in amidases with up to 80% identity with SEQ 2 are described in the prior art.

Box No. VIII **Certain observations on the international application**

Claims 9 and 10 are not correctly dependent since they relate in fact to claims 1-8, but are not dependent on the latter. In addition, they relate respectively to claims 2 or 1 as well, which do not, however, contain the sequences in question that are to be deleted. This is unclear within the meaning of PCT Article 6. In addition, if SEQ 1 and/or 2 were to be fully deleted and the claims were still to refer to claims 1 or 2, these claims would merely mean amidases, as is shown exemplarily hereinafter. For example, claim 9 would relate to an amidase which originally contained SEQ 1 but which is now fully deleted, e.g. only an amidase remains. This is not only unclear within the meaning of PCT Article 6, it is not novel either (PCT Article 33(2)). The same applies to sequences SEQ 1 or SEQ 2 which are partially deleted. Imagine one were to take an amidase containing SEQ 1 in the N-terminus, e.g., IHMPDPDAV, and partially delete I, H, M, D, D, A, V; what would be left would be an amidase with an N-terminal sequence PP. D2 describes an amidase containing the sequence PP N-terminally in positions 6 and 7. This unclear claim 9 would therefore not be novel over D2. The same applies to claim 10.

The term "homology" that is used in the claims is unclear since it does not define any identity to sequences and therefore leaves a very broad scope for interpretation, especially since no method is given for assessing this homology, e.g. what kind of homology table is ultimately used. In this regard, attention is drawn to the fact that the enzymes and genes of the application were compared with D1-D3 in the present report for identities.

Box No. VIII **Certain observations on the international application**

Depending on the program and model, corresponding homologies are correspondingly higher, or at least equivalent to the identity (in the case of SEQ 2, if there were a 100% homology with D1, since the amino acids that are not identical, e.g. M8 and V10, are homologously exchanged M8Q and V10I).

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Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment* on _____

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

The sequence listing in the description, pages 1-4 as originally filed.

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*